FINAL REPORT

of a project entitled

TISSUE THRESHOLDS OF HYPERTHERMIC INJURY IN ARCTIC MAMMALS

Supported in part by

Subcontract No. ONR-418 from the
Arctic Institute of North America

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April, 1972

Introduction

A prior study of oxygen consumption rates of assorted tissues of various arctic mammals produced a sizable fund of basic data on the effects of temperatures between 10° and 45° on tissue respiration in whale, walrus, bearded seal, caribou, ringed seal, arctic fox, marmot, arctic ground squirrel, brown lemming, tundra vole, and redbacked tundra vole (see Fisher: Final Report on the Rate of Oxygen Consumption in Various Tissues of Arctic Animals, Task No. NR 307-245 and A.I.N.A. Subcontract No. ONR-246, 1960). That study was conducted to determine if any differences in metabolic response to temperatures might be detected between marine and terrestrial mammals whose habitual environmental temperatures were different. No such differences were revealed. The most consistent positive results of that investigation were that in each animal kidney always showed the highest respiratory rate, followed in decreasing order by brain, liver, myocardium and dental pulp, and that the tissue respiratory rate was inversely related to body mass. The general character of the temperature coefficients of the various tissue respiratory rates for 10° intervals between 10° and 40° seemed quite similar. However, at 45° mean QO_2 values were higher than at 40° for some tissues and lower for others but no consistency of response could be related to specific animals or tissues.

In planning the present investigation it was hypothesized that if tissue respiratory measurements were made at normal body temperature after tissue samples had been exposed briefly to hyperthermic temperatures between the upper limit of the normal biokinetic range and that at which respiratory enzymes are inactivated, a temperature might be identified beyond which evidence

of metabolic injury could be measured. It is assumed that the rate of oxygen consumption is a reliable general index to the level of metabolic activity in mammalian tissue. It is also assumed that depression of the oxygen consumption rate following experimental modification of a tissue is expressive of metabolic derrangement or injury. It is further assumed that the amount of respiratory depression is a measure of the degree of injury to oxidative metabolic mechanisms which are so fundamental to energy production and cellular survival. It was reasoned that the critical temperature marking the lower hyperthermic level of tissue injury might be different for marine and terrestrial mammals because the circumstances of their environmental temperatures and their special adaptations for regulating body heat are different.

Personnel

The work on this project was accomplished by Alton K. Fisher, Clayton L. Shalla and Marcelia C. Fisher. All three were actively engaged in the effort during the summer of 1969 and 1970. Only Fisher and Fisher were able to continue the work during 1971. The activity in 1971 was devoted to the study of polar bear tissue for slightly less than a month during the spring, and to caribou, wolf and walrus tissue during mid-summer.

The nature of the investigative method required varied, extensive, detailed laboratory manipulations which taxed the physical capabilities of all participants when fresh tissue became available. The unavailability of Clayton L. Shalla in 1971, therefore, significantly reduced the accomplishment for that period.

Facilities

The field work of this project was centered at the Naval Arctic Research Laboratory at Point Barrow, Alaska. Laboratory No. 108-109 was assigned for its use and this was soon equipped to meet the special needs of the program. The most crucial item of required equipment was a respirometer with an adequate supply of manometers and reaction vessels. Although a Gilson differential respirometer had been ordered by N.A.R.L. early in 1969, its delivery date was uncertain. Since a conventional Warburg apparatus was available at the Laboratory, and because the condition of stored glassware, previously used by other workers, is often questionable in many institutions, the principal investigator shipped a generous supply of glassware, valued at $1260.00 to N.A.R.L. to be used if needed. Fortunately, the Gilson respirometer, which is the source of fewer frustrations when operating properly, arrived before the older equipment was required. The Warburg manometers that had been shipped as stand-by equipment were contributed to the stock of the Naval Arctic Research Laboratory.

Other principal items of equipment were an electric drying oven, 4 small serological waterbaths with adjustable temperature controls, a double pan analytical balance, a Cahn electrobalance, trip balances for weighing reagents, and an electric timer.

The Naval Arctic Research Laboratory provided boats and operating crews, as well as an airplane and pilot, and Eskimo hunters, to collect the animal material required in this investigation. It was understood that the use of these transportation facilities and staff personnel necessarily had to be integrated with the many other demands for them imposed by the requirements

of other projects supported by N.A.R.L., but the cooperation was excellent. The time lost because of bad flying weather and adverse ice conditions is an unavoidable ingredient of research in the Arctic. Naturally, not all hunting efforts were successful. In all instances one of the investigators accompanied the hunting party to excise the required tissues, and properly preserve them in cold Krebs-Ringer-phosphate solution for transportation back to the laboratory without delay.

The Fishers were assigned to comfortable family quarters at N.A.R.L. and Shalla was accomodated in the N.A.R.L. dormitory.

Material

The pursuit of this project was guided by the desire of all project personnel to limit the killing of all types of animals to the minimum numbers required by the objectives of the investigation. Needless duplication of animal deaths was avoided by working for extended periods when tissue became available in order to complete as many experiments as possible, and by collaborating with other projects or activities in using tissues not required for their purposes.

The animals used in this study and in the approximate order of their collection were:

2 Ringed seals	165 tissue samples
3 Harbor seals	187 tissue samples
2 Bearded seals	132 tissue samples
7 Brown lemmings	187 tissue samples
5 Collared lemmings	110 tissue samples
2 Polar bears	44 tissue samples
1 Caribou	110 tissue samples
1 Wolf	88 tissue samples
1 Walrus	132 tissue samples

The data previously obtained by Fisher (Final Report ONR-246, 1960) on QO_2 values for kidney, brain, liver, myocardium and dental pulp from various arctic mammals were extremely useful guides for the purpose of evaluating the measurements obtained in the present study. Where prior data for a particular tissue were not available an effort was made to obtain them as the first

step of the study. This was attempted for skin and palatal mucosa of ringed and harbor seals, bearded seal, polar bear kidney, liver and palatal mucosa, and caribou skin and palatal mucosa. Unfortunately, where this was done in the case of polar bear kidney, liver, and palatal mucosa and caribou skin and palatal mucosa, the subsequent lack of time and material prevented acquisition of thermal injury data for those tissues.

Lemming tissues were obtained through the cooperation of Richard V. Andrews and G. Edgar Folk, Jr. who were using these animals in their respective projects at N.A.R.L. Polar bear tissues were secured by enlisting the cooperation of licensed hunting guides who did not object to being followed by a Naval Arctic Research Laboratory airplane during their hunts and whose clients consented to excision of the desired tissues after a bear had been killed. Several research projects at N.A.R.L. shared in the investigation of various parts of a single wolf sacrificed from the animal colony of N.A.R.L. In most instances, the edible parts of all animals that were acceptable for human consumption were divided among the participating Eskimo hunters after the scientific samples were removed. The polar bear meat was given to the people in Barrow. Most of the seal and bearded seal meat served as food for the animal colony.

Research Method

As soon as the animal was killed the desired tissue was excised and placed in chilled Krebs-Ringer-phosphate solution to reduce its metabolic activity and transported to the laboratory with the least possible delay. There the tissue was trimmed into smaller blocks of more appropriate size and transferred to beakers of chilled fresh Krebs-Ringer-phosphate solution and placed in the refrigerator to be used as needed during the next few hours. Preparation of tissue samples was accomplished with the aid of a Stadie-Riggs tissue slicer that yielded slices about 0.5 mm thick. As each slice was removed from the tissue block it was placed in a perforated teflon basket in a numbered beaker filled with cold Krebs-Ringer-phosphate solution until each of the 11 beakers in the series contained a slice sample. The beaker number and the basket number was also the sample number.

Sample numbers 3, 6, and 9 were used as controls because they were evenly spaced throughout 11 samples that were consecutive slices of an anatomic continuum. These control samples were transferred to the reaction vessels without any further treatment. Experimental samples 1 and 2 were immersed for 5 minutes in a beaker of Krebs-Ringer-phosphate solution maintained at 44°C in a serological water bath. Experimental samples 4 and 5 were immersed for 5 minutes in a similar solution maintained at 48°C in a second water bath. Experimental samples 7 and 8 were immersed for 5 minutes in a similar solution at 52°C. Experimental samples 10 and 11 were immersed in a similar solution at 56°C. This was almost a simultaneous operation since 2 laboratory workers could transfer the total of 8 samples in their respective perforated teflon

baskets from the cold solution to the heated solutions with little delay. At the end of 5 minutes of hyperthermia, indicated by a laboratory timer, the experimental samples were as quickly returned to the chilled suspending fluid, effectively terminating the hyperthermic state.

Next, each slice sample was transferred to a 15 ml. Warburg reaction vessel marked with a corresponding number and containing 5 ml. of Krebs-Ringer-phosphate solution with 0.2% glucose. Then 0.3-0.5 ml. 10% KOH solution was added to the center well and a prepared paper wick was inserted. The loaded reaction vessels were attached to Gilson differential manometers which were also numbered consecutively from 1 through 11. The insistence on numbering all samples and containers and manometers insured that mistaken identification of tissue or the data would be rare. The flask-manometer systems were flushed with oxygen for several minutes and the systems were equilibrated in the water bath of the respirometer which was set at 38°C. After equilibration for 20 minutes the manometers were closed, the meniscus of the manometer fluid columns were adjusted to the reference lines, the timer was started for a 1-hour run and all of the syringe micrometer readings were recorded.

At the end of the 60-minute measurement period, the shaking motor was stopped, the manometer fluid meniscus was returned to the reference line in each system, the second set of syringe micrometer readings was recorded, internal pressures were equalized, the systems were opened and then raised from the water bath. The tissue sample from each numbered reaction vessel was placed in a pre-weighed aluminum foil basket marked with a corresponding number which was, in turn, placed in a wire mesh basket that was placed in a

drying oven maintained at 108°C. Samples were dried for at least 18 hours before dry weights were determined.

The oxygen quotient for each sample was calculated as the microliters of oxygen consumed per milligram of dried tissue per hour.

In a few instances as indicated previously, in which no previous basic data were available on the respiratory response of specific tissue to temperatures between 10° and 45°C, the first efforts with those tissues was devoted to obtaining those data. This was accomplished with the direct method of Warburg as described above by placing the tissue samples in the respirometer without any prior experimental treatment and with the water bath temperature initially at 10°C. After completion of one hour of manometric observation, the systems were opened. The water bath temperature was raised to 20°C, the systems were re-equilibrated and then the second hour of respiratory measurement was accomplished. This process was repeated for each next higher temperature required except that the observational period was reduced to 30 minutes for the measurements above 30°C. This method was used extensively in our studies of 1958-59 (see Fisher: Final Report, 1960).

It was believed that since kidney tissue was the most active of any of the tissues previously examined, it also might be most likely to show marked degenerative changes if its metabolic measurement was delayed too long. Therefore, it was always investigated first when several tissues were available and when time permitted completion of manometric observations of the other tissues within 12 to 15 hours from the time the animal was killed. Under these conditions liver was studied next and the integumentary structures last.

When reduction in the number of available personnel in 1971 made it impossible to follow that procedure, kidney tissue continued to be studied as soon

as possible after each animal was killed, and the other tissues were refrigerated at 2°C in Krebs-Ringer-phosphate solution. Liver was examined approximately 24 hours later, palatal mucosa about 48 hours later and skin about 72 hours after the animal had been killed.

These apparently excessive delays were inevitable because the respirometer could accommodate only 11 samples at one time, and the time required to complete a full cycle of the technique was approximately 3 hours. After completion of 4 cycles, at most, all glassware was soiled and it had to be rendered chemically clear again and dried. The justification for using stored tissue stemmed from encouraging results with polar bear liver and palatal mucosa and the irregular and unpredictable acquisition of fresh tissue.

Results

In all, 1714 oxygen quotient determinations were attempted during the course of this project. Some attempts ended in failure because of technical difficulties, such as accidental contamination of individual samples with KOH solution, occasional leakage of manometers, and rare manometer "blow-backs" resulting from improper manipulation of equipment by overly fatigued personnel who remained at their tasks too long. However, the total number of losses were relatively small.

The new basic data on the effects of temperature on the QO_2 of various tissues not previously studied are presented in Table 1. These mean values fit nicely with the relationships described in the Final Report on Subcontract ONR-246, 1960, by Fisher.

These studies were extended on the tissues of Polar Bear No. 2 by additional measurements, begun 21 and 44 hours after the animal was killed, on tissue that had been stored in Krebs-Ringer-phosphate solution at 2°C. The results are presented in Table 2. They suggest that kidney does, indeed, degenerate significantly when stored, and that tissue damage occurs when polar bear kidney is incubated at 45°C. In contrast, polar bear liver is more active at 45° than at 40°C and its activity is unimpaired by storage at least up to 44 hours. This latter finding in the spring of 1971 encouraged further attempts during the summer of that year to utilize stored liver, and integumentary tissue when they could not be studied on the day the animal was killed.

Casual examination and attempts at comparison of the QO_2 values presented in Tables 1 and 2, for example, will illustrate the difficulty inherent in

ready interpretation of more extensive tabulations. This difficulty arises from the different relative oxygen consumption rates of various tissues from the same animal, and the differences of oxygen consumption rates of similar tissues from different animals because of the influence of body mass, as well as variations attributable to age, sex, activity and state of health. In order to circumvent this difficulty as much as possible and to reduce all experimental data to more readily comparable terms, all subsequent tabulation of experimental results in this report will be in terms of Quotients of Relative Activity based on QO_2 determinations for 38°C. The QRA is essentially a percentage value expressive of the QO_2 of the experimental tissue in relation to the control tissue which is arbitrarily assigned a relative value of 1.00:

$$QRA = \frac{QO_2 \text{ of experimental tissue} \times 100}{QO_2 \text{ of control tissue}}$$

It will be noted in the following Tables that the results for harbor and ringed seals are calculated together. This is done because no significant differences could be observed between the QO_2 values for the tissues of the two species, and the effects of hyperthermia were similar in both. The advantage of pooling the data rested in creating a larger statistical series that facilitated a more general characterization. The same treatment was accorded the data for brown and collared lemmings, and for the same reasons.

The effects of 5 minutes of prior hyperthermia on the respiration of kidney slices at 38° are shown in Table 3. The general quality of hyperthermic effects is similar in that progressively higher temperatures have correspondingly greater depressant effects on subsequent respiration. However,

species differences cannot be detected with any degree of clarity until the results for 56° are examined. When exposed to that temperature, which is near the inactivation point for cytochrome oxidase if sufficient time is allowed, the effect is magnified, and differences between the various types of animals become more readily apparent. It should be indicated again that all kidney samples were as fresh as the method of investigation permitted so that reasonably reliable results might be anticipated.

The effects of 5 minutes of prior hyperthermia on liver slices are shown in Table 4. Why 44° should have a more depressant effect on lemming liver than 48° is not explained but it was apparent in both species. The results for stored walrus liver must be rejected because the control QO_2 was lower than that previously reported (Fisher, 1960) for fresh tissue, and all experimental hyperthermic temperatures had somewhat similar effects on its residual respiration. It is quite possible that the time involved in "landing" and butchering so large an animal permitted hyperthermia to develop in this massive organ before tissue samples free of degenerative change could be obtained. The influence of storage of walrus, caribou and wolf liver for 24 hours at 2°C is unknown. The control QO_2 of caribou liver was slightly higher than that previously reported, suggesting that it had retained its original level of vitality. Since no prior data on wolf tissue were available, its behavior under experimental conditions cannot be explained satisfactorily. It could have degenerated as a consequence of refrigerated storage. On the other hand, it may be significant that its pattern of respiratory deterioration caused by hyperthermia resembled at least superficially that of wolf kidney by a reduction of their rates of deterioration between 48° and 52°. If these

somewhat parallel effects are real, the data for wolf liver becomes acceptable.

The experimental results for skin and palatal mucosa, which include fewer species, are shown in Tables 5 and 6, respectively. The data for lemming skin are unreliable because the few samples of this tissue which were measured yielded widely scattered QO_2 values. The cause probably was trauma inflicted by shaving the hair after the skin had been separated from the carcass. These examples of integument are notable for their similarly low oxygen quotients and for their apparent relatively greater resistence to injury from exposures to temperature of 44° and 48°. The stimulant effect of 48° on the respiration of palatal mucosa was first noted in seal tissue and was thought to be either a technical or statistical artifact. However, extensive enlargement of the sample series could not change the result.

The results for walrus dental pulp are shown in Table 7 and must be regarded as unreliable. It had been stored for 48 hours prior to respirometry. At that time the QO_2 of the control samples was approximately one-half of that previously obtained for fresh tissue. Moreover, the atypical effects of hyperthermia might suggest that this tissue is unusually resistant to thermal injury but this is not supported by other studies on bovine dental pulp.

Discussion

Perhaps the best illustration of the actual and relative effects of hyperthermia on the subsequent QO_2 of visceral and integumentary structures is provided by the mean data for seal tissues which were studied more than those of any other animal. Figure 1 depicts the actual depression of the oxygen consumption rates of kidney, liver, skin and palatal mucosa as the result of prior heating. Because of their greatly different normal respiratory rates the deterioration curves may suggest that kidney is vastly more sensitive to thermal injury than the other tissues. But when the same data are converted to QRA values and similarly plotted, inspection of Figure 2 shows the effects for any single temperature are not as different as at first seemed apparent. Nevertheless, in terms of relative sensitivity to hyperthermia, kidney does indeed seem most susceptible to this type of injury, followed by liver, skin and palatal mucosa.

Whether or not the greater sensitivity of kidney tissue to heat injury is attributable to its relatively higher oxygen consumption rate is not readily answered. If this should prove to be true, then kidney cortex would be an excellent index tissue to be used when testing for species susceptibility to thermal injury. Inspection of Figure 3 will show that it is difficult with most of the animals studied to show clear differences in the effects of exposure to 44°, 48° and 52°. It is only when the more drastic influence of 56° is imposed that distinctions become more clear. The results of that temperature on the respiration of kidney slices for the different mammals are shown in Figure 4. The marine mammals, as a group, show a greater depression of respiration than the terrestrial mammals. The influence of body mass within each

group is not clear with respect to the marine mammals but is suggested by the results for the terrestrial mammals.

The results of the studies on liver slices, although seeming to reflect characteristics similar to those of kidney, must be viewed with caution until more data are available. The effects of storage on the respiratory behavior of those species of liver which were so treated must be established before the results obtained from such material can be more fully evaluated. Insufficient information has been gathered for skin and mucosa to warrant any judgements about species differences.

No thresholds of thermal injury have been identified for kidney or liver. Inasmuch as all QRA values for these tissues were below 1.00 it is assumed that injury would have been produced at some temperature lower than the lowest experimental temperature employed, namely, 44°C. By way of contrast, the respiration of skin and palatal mucosa does not deviate far from normal until temperatures higher than 48°C are involved. It would appear that in the instance of these integumentary structures no sharply definable threshold temperature exists, but rather, there is a range of temperature which divides thermal injury from thermal tolerance. This might be attributable to individual variation within a species. Under this circumstance the concept of a threshold of thermal injury becomes a generality which is unsuited for precise analytic purposes and one is compelled to rely for comparisons upon relative degrees of injury inflicted by a single standard temperature that is not high enough to completely inactivate crucial respiratory enzymes within the time of the test period. This provision was a part of the present study.

Tentative explanations of the biologic foundations of the differences in thermal tolerance between the marine and terrestrial mammals are intriguing. Is it possible that the marine mammals have in their aqueous environment a more ready facility for the transfer of excess body heat so that adaptations to hyperthermia are slightly less important than in the terrestrial mammals?

Conclusions

While this project was pursued essentially as it was originally designed, the experience that was acquired has shown a more direct approach to its objectives. Any further studies of this sort, intended only to detect species levels of susceptibility to thermal injury, might use a single experimental temperature near 56°C. The advantages are simplicication of technique, maximum biologic effect, and enlargement of the fund of useful observations by concentration on the most diagnostic test temperature.

The results of this project indicate that the tissues of terrestrial mammals, as a class, seem better qualified to tolerate hyperthermia than do those of the marine mammals. However, since this study is probably the first of its kind and the data are relatively limited, this work should be extended until the facts are securely established. The implications of these results, if corroborated, may be important to management of marine mammals in captivity. Moreover, they may indicate precautions to be observed when working with these animals in their wild state but out of their aquatic environment.

The results of this study raise questions of especial interest to physiology and biochemistry. Are the alleged differences acquired or genetically determined? In either case, what are the dynamics of enzymatic adjustments that are involved?

Table 1

Effect of Temperature on Tissue Respiration

Animal	Tissue	Mean QO_2					
		10°	20°	30°	35°	40°	45°
Seal	Flipper skin	0.15	0.36	0.79	1.39	1.62	1.69
Seal	Palatal mucosa	0.11	0.20	0.53	0.93	1.33	1.18
Polar bear	Kidney	3.40	4.50	7.19	7.92	11.20	8.76
Polar bear	Liver	0.25	0.56	1.03	1.70	1.90	2.34
Polar bear	Palatal mucosa	0.12	0.15	0.48	0.94	0.96	0.74
Caribou*	Skin	0.11	0.15	0.50	0.73	0.89	0.79
Caribou**	Palatal mucosa	0.24	0.29	0.85	1.17	1.44	1.47

* Stored for 72 hours at 2°C.

** Stored for 48 hours at 2°C.

Table 2

Effect of Storage on the QO_2 of Polar Bear Tissue in Krebs-Ringer-phosphate Solution at 2°C.

Tissue	Hours After Kill	10°	20°	30°	35°	40°	45°
Kidney	4½	2.92	5.42	7.40	8.02	11.28	7.50
Kidney	21	-	4.32	7.62	8.21	9.31	6.20
Kidney	44	2.05	2.76	5.97	8.47	8.01	5.98
Liver	4½	0.26	0.69	1.08	1.62	1.76	2.34
Liver	21	0.46	0.53	1.17	1.34	2.84	3.08
Liver	44	0.47	0.53	0.90	1.41	2.41	3.35
Palatal mucosa	4½	0.13	0.16	0.61	1.13	1.13	0.58
Palatal mucosa	21	0.12	0.09	0.86	0.94	1.00	0.67
Palatal mucosa	44	0.19	0.33	0.74	1.29	1.26	1.16

Table 3

Residual Respiration in Kidney Slices

After 5 Minutes of Prior Hyperthermia

Animal	Mean QO_2 of Controls	QRA After 44°	QRA After 48°	QRA After 52°	QRA After 56°
Walrus	11.22	.84	.77	.63	.24
Bearded seal	7.02	.89	.76	.53	. 8
Ringed & harbor seals	12.59	.94	.84	.56	. 9
Caribou	16.07	.88	.91	.77	.40
Wolf	17.35	.89	.65	.59	.34
Brown & collared lemmings	15.88	.91	.74	.47	.25

Table 4

Residual Respiration in Liver Slices

After 5 Minutes of Prior Hyperthermia

Animal	Mean QO_2 of Controls	QRA After 44°	QRA After 48°	QRA After 52°	QRA After 56°
	Fresh Tissue				
Ringed & harbor seals	7.46	.93	.79	.63	.20
Brown & collared lemmings	13.20	.80	.91	.73	.24
Bearded seal	2.08	.90	.92	.82	.33
	Tissue Stored at 2°C For 24 Hours				
Caribou	1.46	.92	.80	.73	.66
Wolf	1.96	.91	.77	.83	.60
Walrus	1.96	.69	.64	.71	.66

Table 5

Residual Respiration in Skin Slices

After 5 Minutes of Prior Hyperthermia

Animal	Mean QO_2 of Controls	QRA After 44°	QRA After 48°	QRA After 52°	QRA After 56°
Bearded seal	1.15	.91	1.00	.80	.16
Ringed & harbor seals	1.60	1.04	.96	.73	.20
Brown lemming*	2.02	.93	.78	.68	.46

* Unreliable because of injury to tissue in preparation of samples.

Table 6

Residual Respiration in Palatal Mucosa Slices

After 5 Minutes of Prior Hyperthermia

Animal	Mean QO_2 of Controls at 38°C	QRA After 44°	QRA After 48°	QRA After 52°	QRA After 56°
Bearded seal	1.61	.89	.97	.79	.15
Ringed & harbor seals	1.16	.98	1.11	.91	.33

Table 7

Residual Respiration in Dental Pulp Slices

After 5 Minutes of Prior Hyperthermia

Animal	Mean QO_2 of Controls	QRA After 44°	QRA After 48°	QRA After 52°	QRA After 56°
Walrus*	0.96	1.03	.89	.89	.60

* Stored for 48 hours at 2°C,

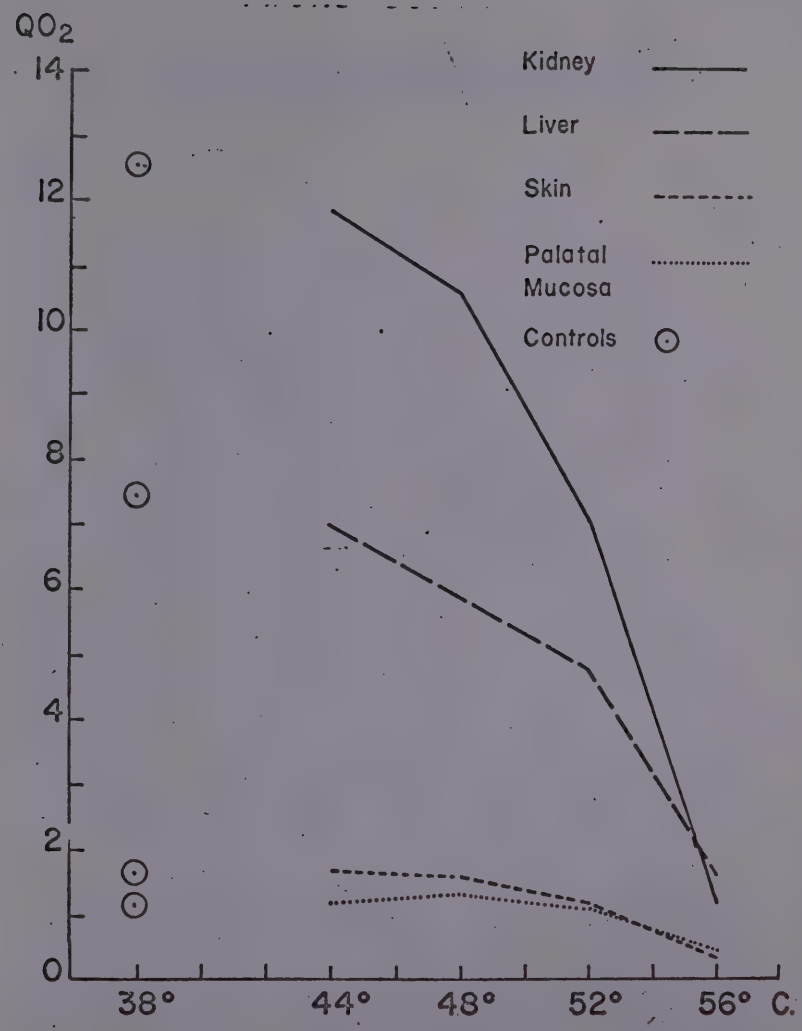

Figure 1, Absolute effects of 5 minutes of prior hyperthermia on respiration in seal tissues.

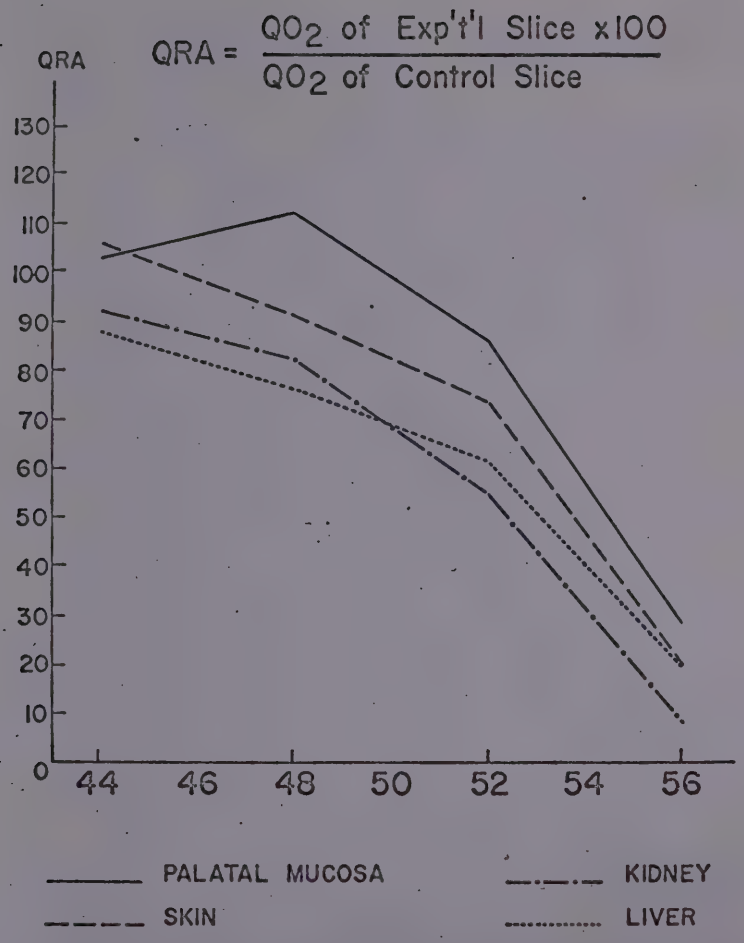

Figure 2. Relative effects of 5 minutes of prior hyperthermia on oxygen consumption rates of seal tissues.

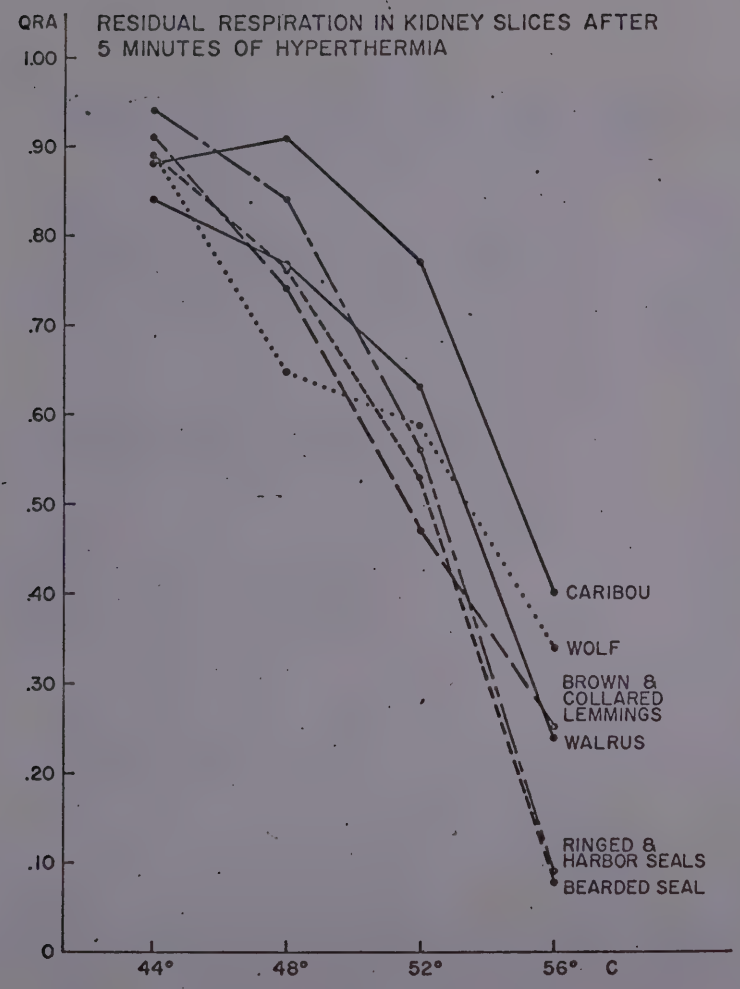

Figure 3. Relative degrees of sensitivity to hyperthermia of kidney of several arctic mammals.

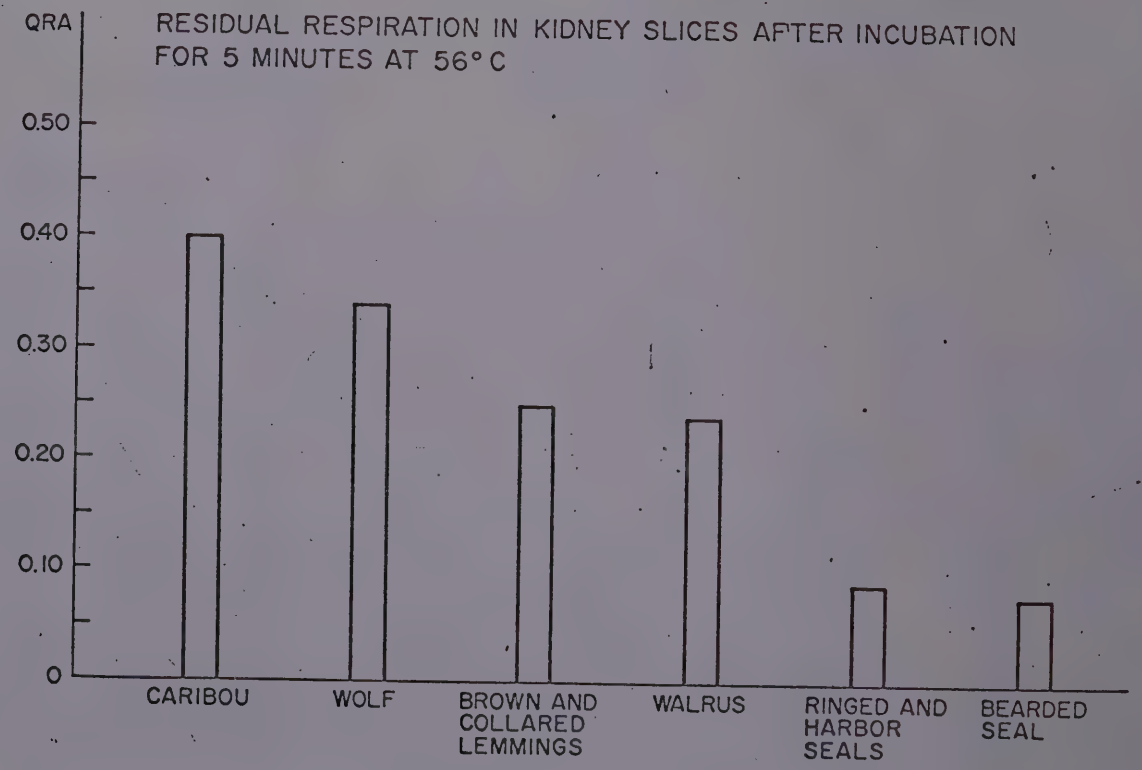

Figure 4. Relative sensitivity of kidney slices from different arctic mammals to exposure to 56°C.

APPENDIX A

Financial Statement

A.I.N.A. Subcontract No. ONR-418

Expenditures:

Round trip airplane tickets between Iowa City, Iowa and Barrow, Alaska:

6-13-69 for A. K. Fisher, C. L. Shalla, and M. C. Fisher	1550.75
6-15-70 for A. K. Fisher, C. L. Shalla, and M. C. Fisher	1494.00
3-22-71 for A. K. Fisher and M. C. Fisher	1085.02
7-15-71 for A. K. Fisher and M. C. Fisher	1108.80
sub-total	5193.57

Per Diem in transit:
Cabs, airport limousines, meals, and lodgings:

1969	346.55
1970	298.27
1971 (March - April)	159.40
1971 (July - August)	230.60
sub-total	1034.82

Supplies:

Stadie-Riggs slicer blades	24.00
10 doz. reaction vessel springs	15.00
Photocopies of research data	5.00
sub-total	44.00

Shipping charges:
For transporting equipment to and from N.A.R.L.:

6-11-69 Packing and shipping charges for 63 Warburg manometers given to N.A.R.L., freight to Fairbanks	37.59
8-21-69 Air freight to Iowa City	77.14
sub-total	114.73

Total expenditures from Subcontract funds $ 6387.12

Funds authorized for A.I.N.A. Subcontract ONR-418:

4-2-69	Original authorization	2986.00	
7-21-71	Supplementary authorization by amendment of subcontract	1980.00	
	Total	4966.00	
			$ 4966.00

Funds received from A.I.N.A.:

AINA check no. 13931 dated 4-28-69	1194.00	
AINA check no. 14005 dated 5-13-69	1493.00	
AINA check, no. not recorded but received at NARL in July, 1970	1584.00	
Total	4271.00	
		$ 4271.40

Balance receivable by subcontractor: $ 694.60

Summary:

Total expenditures	6387.12
Total funds authorized	4966.00
Funds provided by subcontractor	$ 1421.12

Allen K. Fisher